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MICROBIAL WATER QUALITY STUDY OF THE OTTAWA RIVER AT LEMIEUX ISLAND, 1984-1985

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ABSTRACT

A 15-month microbiological study at one station on the Ottawa River was undertaken to evaluate seasonal fluctuations and the potential of using coliphage as a water quality indicator. Results showed that during the September-December period fecal coliform guidelines were regularly exceeded in this section of the river.

Coliphage counts were found to be closley related to fecal coliform and E. coli populations. Ratios between E. coli and coliphage varied between 2.2:1 to 3.9:1 depending on the sample grouping.

EXECUTIVE SUMMARY

This 15-month study was carried out to evaluate seasonal fluctuations in microbiological water quality of the Ottawa River at one station and to evaluate the potential of coliphage as a water quality indicator. Weekly samples were collected over a 15-month period, September 1984 to December 1985, and tested for fecal coliforms by MF and MPN procedures, E. coli MF, fecal streptococcus MF and coliphage. The results showed that during both September-December periods, fecal coliform counts frequently exceeded provincial guidelines and rarely exceeded the guidelines during the remainder of the year. Similar findings were also noted in a 1971 study.

Based on the statistical analysis of the coliphage and bacterial data and the fairly consistent ratios observed between fecal coliforms, E. coli and coliphage, it would be feasible to set a coliphage water quality guideline of 20 coliphage/100 mL.

The simplicity, speed and inexpensive nature of the coliphage procedure, coupled with the stability of coliphage in water samples, are major factors for including this test in all recreational water quality surveys. With a sufficient Canadian data base, the procedure could be initially used in parallel with traditional indicators (fecal coliforms, E. coli, fecal streptococci) and eventually replace these microbial indicator populations of potential health hazards due to fecal contamination in recreational waters and drinking water source waters.

INTRODUCTION

A study to evaluate seasonal fluctuations in the microbiological water quality of the Ottawa River was carried out September 25, 1984 to December 4, 1985. Water samples were collected weekly from the Ottawa River, at a Water Quality Branch - Ontario Region monitoring station located on Lemieux Island adjacent to the Ottawa-Carelton water filtration plant, where a pumping station for sample collection had been established previously by Inland Waters Directorate personnel.

This project was also seen as an opportune means of assessing the use of coliphage as an indicator of water quality in a northern Canadian river and to study the relationship of coliphage to traditional indicator microbial systems.

Guelin in 1948 was the first researcher to properly apprise the potential of bacteriophages as indicators of fecal pollution. Since Guelin's recognition of the potential of bacteriphages to act as indicator systems, there have been several research reports indicating the potential of bacteriophage/coliphage to act as indicators of bacterial water quality; i.e. Busco 1963, Kuznetsova and Ostrowskaja 1963, Amin-zade and Poultof 1964, Kenard and Valentine 1974, Scarpino 1975, Zais 1982, and Wentsel R.S., O'Neil and Kitchens 1982 and Kennedy et al. 1985; and viral water quality, i.e. Vaughn and Metcalf 1975, Kott, et al., 1978, and Grabow et al. 1984.

The most detailed and intensive studies on growth and recovery of coliphage can be found in the Atlantic Research Report of 1979 by Scott, O'Neal, Wilkinson and Kitchens. In an earlier major review of coliphages by Scarpino 1975, he stated "Correlations appear to exist in fresh and marine waters between fecal bacterial pathogens, such as Salmonella and Shigella species and fecal indicator bacteria, such as Eschericia coli and their bacteriophage. Then in 1984, Grabow et al. reported "coliphage counts could give a useful estimate of numbers of other microorganisms in sewage polluted water", and in their studies "evidence is presented that, even though counts of coliphages may not always directly correlate with those of enteric viruses, coliphages meet the basic requirements of an indicator for the virological safety of water".

From the studies performed at the Atlantic Research Corporation (1979) and others reported in the recent literature, it would appear that in the various environmental and drinking waters tested that the coliphage procedure is a reliable indicator of E. coli and coliforms. There is also sufficient evidence to suggest that the coliphage test has many advantages over traditional bacteriological and virological tests in that the procedure is economical, simple to perform and provides results within six hours of testing. The speed with which results can be obtained indicates that the coliphage test is a desirable technique where approximate or hazard estimate data are required as soon as possible, i.e. (1) in the repair of broken water

mains where an indication is required to determine whether fecal pollution organisms have entered the line, (2) in cases of suspected contamination of enclosed water supplies such as found onboard cruise ships, or (3) in mobile field studies to test, characterize or give priority ratings to potable water sources.

Although a review of the literature on the coliphage test indicates that it may be an ideal test for approximation of health hazard estimation due to fecal pollution, there appears to be a reluctance to accept research results and conclusions, and directly apply test result implications to local waters, even though the procedure has now been tentatively accepted by North America's two major method standardization organizations ASTM and APHA. It may therefore be necessary for each area or jurisdiction considering the use of the coliphage indicator to establish a relationship to coliforms and other traditional indicators and pathogens (even though there is no direct numerical relationship between coliforms, fecal coliforms, E. coli and the degree of hazard as related to the incidence and infectivity rate of Salmonella, Shigella, cholera, rota viruses and other viruses). Also, there is no consistent and obvious numerical relationship in receiving waters and drinking waters between fecal coliforms, E. coli, Salmonella, Shigella, Cholera, viruses and coprostanol, the absolute indicator of fecal contamination (Dutka and El-Shaarawi 1975).

In all uses of indicator organisms, we are dealing with a concept; a concept that usually works and is protective (and possibly

over protective) of users of potable and natural waters. Due to increasing stress on water supplies, rising analytical costs, and contamination resulting from urban and rural runoff, untreated waste discharges and natural disasters which require immediate answers (i.e. earthquakes, volcano eruptions, frost upheaval of pipes), we must develop cheaper, simpler and quicker indicator systems which are stable over a 30 to 60 hr transport period and will reflect both bacterial and viral contamination from sewage. Coliphages appear to be the obvious candidates. However, to allay the doubts of local implementers of this indicator system and those involved in guideline setting, it would be prudent to collect more local data from potential application sites as well as cross country and marine data to support the use of the procedure.

Microbiological data from this 15 month study are presented and evaluated below.

METHODS

Sample Collection

Water samples from September 25, 1984 to December 4, 1985 on a weekly basis were collected by pump from a pipe secured 1 metre above the bottom and approximately 15 metres north of Lemieux Island in the midstream of the Ottawa River. After the pipe was flushed for at

least one hour, the microbiological samples were collected, placed on melting ice in an insulated chest and sent by courier to the laboratory, where it was processed within 24 hours of collection.

Microbiological Tests

Fecal coliform densities were estimated by the membrane filtration and most probable number five-tube dilution techniques. The membrane filtration procedure used is detailed in the Department of Environment Inland Waters Directorate manual "Methods for Microbiological Analysis of Waters, Wastewaters and Sediments (1978)"; the medium used was FC agar. The sensitivity of this MF technique is such that it is theoretically possible to enumerate one fecal coliform in 100 mL of water sample. Studies by Dutka, Kuchma and Kwan (1979) have shown however that in reality, due to various stresses, this procedure usually measures 6 to 26% of the potential population present.

Investigations of the five-tube, three to four decimal series (10, 1.0, 0.1 and 0.1) most-probable-number (MPN) procedure with A1 broth (Dutka 1978) have shown that this medium is fairly specific for *E. coli* with over 90% of the enumerated population being *E. coli* (Dutka *et al.* 1979). The sensitivity of the technique is such that if a healthy fresh laboratory population is estimated, the count reported is between the 95% confidence limits (APHA 1985). However, with

environmentally-stressed populations, subjected directly to a stressful incubation temperature of 44.5°C, it is estimated that a minimum of 40% of the true population is enumerated (Dutka et al. 1979).

E. coli densities were estimated by the membrane filtration technique using mTEC agar and with the membrane filters being transferred to pads saturated with urea substrate, as detailed by Dufour, Strickland and Cabelli (1981). In fresh waters, it has been reported that 87-91% of the population enumerated (Dufour et al. 1975, 1981) by this procedure were E. coli. The sensitivity of this MF technique is such that it is theoretically possible to isolate one E. coli from 100 mL of water sample. However, studies by Dutka, Kuchma and Kwan (1979) have shown that, in reality, membrane filter procedures measure 5 to 90% of the potential population depending on the stress state of the organisms being enumerated and the membrane filter used (Tobin and Dutka 1976).

Fecal streptococci populations were estimated by the membrane filtration technique using KF agar and an incubation period of 48 hours at 35°C. The sensitivity of the MF technique as stated earlier, is such that one fecal streptococcus can be isolated from 100 mL of water, however, there does not appear to be any documentation on what percent of the actual fecal streptococci sample population is actually enumerated. Based on fecal coliform MF estimates and depending on the brand of membrane filter used (Tobin, Dutka 1979), we suspect 25 to 90% of the actual viable population is estimated.

Coliphage are bacterial viruses (bacteriophage) which infect and replicate in lactose fermenting, Enterobacteriacae (coliforms and fecal coliforms). Since coliphage replicate only in coliform and fecal coliform organisms, the finding of coliphage in water samples also indicates the possible presence of these indicators and pathogenic organisms. The procedure used, in these studies to estimate coliphage concentrations, is similar to that found in section 919C of the 16th edition APHA Standard Methods 1985. This procedure can theoretically detect one coliphage, (1 coliphage in 100 mL of water sample) where water turbidity is not in excess of 25 NTU. Waters with excess turbidity may show reduced coliphage counts in excess of 90%, due to the ready adsorption of phage to particles and thus removed from the water phase.

RESULTS AND DISCUSSION

Table 1 presents a summary of all the data collected during the September 25, 1984 to December 4, 1985 period. In reviewing these data, it is advisable to remember that, in 1971, a guideline was adopted by the Province of Ontario for recreational waters that included a bacteriological standard of 100/100 mL for fecal coliforms and 20/100 mL for fecal streptococci. The fecal coliform guidelines are similar to those of other nations, e.g. Italy <100, U.S.A <200, Belgium <200, West Germany <100, Denmark 200, Sweden 100, France <200

and EEC bathing water recommendation is 100/100 mL. In Table 1, it can be seen that the provincial standard for fecal coliforms was exceeded 18 times when tested by the membrane filter (MF) or most-probable-number (MPN) techniques. The highest FC population estimate was made by the MPN procedure 540/100 mL (November 5, 1984) while the next highest estimate 360/100 mL (November 13, 1984) was made by the MF procedure. The highest E. coli MF counts 190/100 mL were found on October 29, 1984 and November 13, 1984.

During the only period where sample collection was replicated, September 24 to December 4, 1984, and September 25, 1984 to December 4, 1985, the following mean counts/100 mL were observed, indicating that the 1985 samples were slightly more polluted than the 1984 samples.

1984 - Coliphage 33.2, F.C. MF 121.1, E. coli 72.0, FC MPN 144.6 and F. Strept 98.3

1985 - Coliphage 26.9 F.C. MF 159.5, E. coli 105.0, FC MPN 255.3 and F. Strept 134.3.

Also during this period of sample duplication, coliphage to F.C. MF, E. coli and FC MPN ratios were: 1984; 1:36, 1:2.2, and 1:4.4 and for 1985; 1:5.9, 1:3.9 and 1:9.5.

It can also be seen in Table 1, that fecal streptococci counts exceeded OME (1968) guidelines of 20/100 mL in 40 of the 55 samples tested; 12 of the non-exceedences were from the January-May period.

Maximum fecal coliform and E. coli counts occurred in the September-October period, while minimum populations were found in the January to May period. Analyses of samples from the rest of the year produced variable counts with occasional highs and lows. This pattern of seasonal fecal coliform densities was also noted on the Ottawa River in 1971 by Blais (1973).

Fecal streptococci densities tended to maximize earlier in the year compared to fecal coliform and E. coli, roughly during the mid-July to mid-October period. With the exception of the January to May period, it would appear fecal streptococci populations are usually greater than 1968 OME guidelines in this part of the Ottawa River.

In 1971 a bacteriological study of the Ottawa River was completed by Blais (1973) and in this study there were three sampling sites near the present Lemieux Island site. One site was slightly upstream of and on the western side of Lemieux Island, and the other two sampling sites were downstream slightly at the Ottawa Rowing Club and at l'Escale. Membrane filter fecal coliform mean counts obtained at these sites were: Lemieux Island 25/100 mL spring, 50/100 mL summer and 70/100 mL fall; L'Escale which is downstream on the east shore had 960/100 mL summer and 210/100 mL fall, while at the Ottawa Rowing Club on the western bank, only a summer mean was obtained of 160/100 mL. Blais' data indicates a downstream degradation of the water quality from Lemieux Island during the early 1970's. However, in comparing the 1984-85 Lemieux Island data with the 1971 data and taking into account that the samples were collected on different shores of the

Island, it would appear that the Ottawa River microbiological health indicator populations in this section of the river have remained similar with maximum population still occurring in the fall. The waters of this section seemed to consistently exceed provincial recreational water quality guidelines, i.e. 100 fecal coliform/100 mL and 20 fecal streptococci/100 mL during the late August to early December period; a period when little recreational use is made of the river at this point, especially contact sports.

To more closely examine the pattern of fecal coliform MF and MPN counts which exceeded the recommended provincial guidelines of 100/100 mL, Table 2 was developed. Ratios between coliphage and fecal coliform/E. coli are also shown. From Table 2 it can be seen that fecal coliform counts exceeded the guidelines of 100 FC/100 mL 18 times by both the MF and MPN techniques in 22 water samples. With the exception of a February 5, 1985 sample, all other samples containing more fecal coliforms than the guidelines occurred during the August 26 to December 4 period. For these samples, E. coli and FC/coliphage ratios varied from 3.6:1 to 8.2:1.

Based on the seasonal trends observed in Table 1, the results of duplicated 1984 and 1985 samples and Table 2 data, Table 3 was prepared showing the mean counts for various periods of time as well as coliphage: fecal coliform and E. coli ratios. One interesting (but commonly observed and noted) phenomenon illustrated in Table 3 is that, depending on the enumeration technique used (MF or MPN and the media and membrane filter type) (Dutka et al. 1979) and the time of

the year the samples are collected, fecal coliform - coliphage ratios vary from a high of 9.5:1 to a low of 2.6:1, both extremes shown by the MPN technique. E. coli/coliphage ratios varied from 2.2 to 3.9.

Based on the mean fecal streptococci and fecal coliform populations (Table 3) and downplaying the January 8 to February 19 period, the fecal streptococci data are very suggestive that there should be minimal concern about human feces (Geldreich 1966) being the main contributor of microbial health indicator populations in these waters.

To assess the statistical relationship between the various parameters and establish firmly the correlation between coliphage and fecal coliforms, a statistical evaluation of the 55 samples was undertaken. The parameters analyzed were as follows: X_1 = coliphage, X_2 = fecal coliforms MF, X_3 = E. coli, X_4 = fecal streptococci and X_5 = fecal coliform MPN. The association between the five water quality indicators is given in the following correlation matrix.

	X_1	X_2	X_3	X_4	X_5
X_1	1.00	0.38**	0.28*	0.15	0.41**
X_2		1.00	0.89**	0.13	0.80**
X_3			1.00	0.29*	0.85**
X_4				1.00	0.37*
X_5					1.00

This matrix gives the correlation between each pair of log parameters. For example, in the first row and second column, we have 0.38 which is the correlation coefficient between log fecal coliform and log coliphage. Values marked by * and ** are significant at the 5% and 1% levels, respectively. Coliphage is highly correlated with fecal coliforms MF and MPN and correlated with E. coli and fecal coliform MPN. E. coli are highly correlated with fecal coliform MPN and correlated with fecal streptococci. Fecal streptococci are correlated with fecal coliform MPN.

To study the total variation in the water systems and determine which of the five water quality parameters have the largest contribution, principal component analysis was used to divide the total variation into five uncorrelated components. The results showed that the first two components contain 78.3% of the total variation. The summary presented below gives the explained variation for each of the five principal components:

	Principal Components				
	1	2	3	4	5
% explained variation	59.8	18.5	16.2	3.8	1.6

The first principal component PC_1 is dominated by fecal coliform MF, E. coli, fecal coliform MPN and coliphage. The second principal component PC_2 is dominated by fecal streptococci and the third principal component PC_3 is dominated by coliphage. The expressions for PC_1, PC_2 and PC_3 are:

$$PC_1 = 0.305\ \ln X_1 + 0.530\ \ln X_2 + 0.533\ \ln X_3 + 0.234\ \ln X_4 + 0.536\ \ln X_5$$

$$PC_2 = -0.006\ \ln X_1 - 0.306\ \ln X_2 - 0.124\ \ln X_3 + 0.944\ \ln X_4 + 0.019\ \ln X_5$$

and

$$PC_3 = 0.942\ \ln X_1 - 0.131\ \ln X_2 - 0.289\ \ln X_3 - 0.073\ \ln X_4 - 0.086\ \ln X_5$$

Modelling coliphage as a function of the other parameters indicated that fecal coliform MPN is the most suitable variable. The model is

$$\text{Coliphage} = 2.452 + 0.2684\ \ln \text{FC MPN}$$

This model is suitable for describing the variability in coliphage as a function of fecal coliform MPN. These results are not unexpected as both coliphage and fecal coliform MPN are broth type measurements and the coliphage host is enumerated by the fecal coliform MPN technique.

Based on the statistical analysis and the fairly consistent ratios observed between fecal coliforms, E. coli and coliphage, it would be feasible to set a coliphage water quality guideline of 20 coliphage/100 mL. The simplicity, speed and inexpensive nature of the

coliphage procedure coupled with the stability of coliphage in water samples (Atlantic Research Corporation 1979) are major factors for including this test in all recreational water quality surveys. With a sufficient Canadian data base, the procedure could be used initially in parallel with traditional indicators (fecal coliform, E. coli, fecal streptococci) and eventually replace these microbial indicator populations of potential health hazards due to fecal contamination in recreational waters.

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TABLE 1 Lemieux Island, Ottawa River, Coliphage-Coliform Study, 1984-1985

Date	Coliphage /100 mL	Fecal Coliforms MF/100 mL	*E. coli* MF/100 mL	Fecal Streptococci MF/100 mL	Fecal Coliforms Al Broth MPN/100 mL
Sept. 25/84	10	120	80	580	-
Oct. 2/84	40	126	102	132	240
Oct. 9/84	30	156	88	138	240
Oct. 16/84	90	152	120	118	350
Oct. 17/84	25	120	96	92	350
Oct. 23/84	15	170	104	128	240
Oct. 29/84	20	240	190	110	180
Nov. 5/84	30	180	128	150	540
Nov. 13/84	25	360	190	51	350
Nov. 20/84	20	130	30	26	70
Nov/27/84	25	62	42	65	79
Dec. 4/84	20	98	90	22	170
Jan. 8/85	5	52	36	5	49
Jan. 15/85	5	64	15	11	46
Jan. 22/85	<5*	82	54	14	49
Jan. 29/85	5	78	74	14	33
Feb. 5/85	<5	135	60	14	110
Feb. 12/85	15	71	33	7	17
Feb. 19/85	<5	72	40	7	13
Feb. 26/85	<5	65	31	40	23
Mar. 7/85	15	65	49	47	33
Mar. 14/85	5	64	45	11	23
Mar. 28/85	25	43	31	20	6
April 4/85	5	50	31	134	49
April 11/85	30	28	15	61	17
April 17/85	60	22	5	17	13
April 24/85	15	47	30	71	49
May 1/85	10	29	12	9	5
May 7/85	5	19	13	10	11
May 16/85	5	12	14	3	8
May 23/85	<5	11	16	140	7
June 12/85	<5	16	12	20	8
June 26/85	<5	33	25	45	-
July 9/85	15	25	21	90	11
July 16/85	10	26	18	210	33
July 23/85	<5	16	13	>500**	23
July 30/85	10	25	22	300	23
Aug. 7/85	10	25	18	400	21
Aug. 13/85	5	28	38	250	70
Aug. 20/85	5	93	57	180	17
Aug. 26/85	10	110	60	195	95

TABLE 1 Lemieux Island, Ottawa River, Coliphage-Coliform Study, 1984-1985 Continued.

Date	Coliphage /100 mL	Fecal Coliforms MF/100 mL	*E. coli* MF/100 mL	Fecal Streptococci MF/100 mL	Fecal Coliforms Al Broth MPN/100 mL
Sept. 4/85	<5	120	93	150	350
Sept. 10/85	5	45	42	420	49
Sept. 17/85	<5	80	75	450	23
Sept. 24/85	10	75	48	120	130
Oct. 1/85	45	230	125	255	170
Oct. 8/85	35	87	74	180	130
Oct. 16/85	35	130	62	93	79
Oct. 23/85	40	170	97	140	240
Oct. 30/85	10	170	105	90	49
Nov. 6/85	40	43	37	80	140
Nov. 13/85	25	170	76	30	350
Nov. 20/85	10	60	34	32	23
Nov. 29/85	45	93	58	18	140
Dec. 4/85	70	104	76	43	140

* For calculation purposes treated as 1.
**Estimated for calculations as 600.

TABLE 2 **Samples where fecal coliform counts, MF and MPN exceeded provincial guidelines. Ratios of mean coliphage to mean fecal coliforms are also shown.**

Date	Coliphage /100 mL	Fecal Coliforms MF/100 mL	Fecal Coliforms MPN/100 mL	*E. coli* MF/100 mL
Feb. 5/85	<5	135	110	60
Aug. 26/85	10	10	95	60
Sept. 4/85	<5	120	350	93
Oct. 1/85	45	230	170	125
Oct. 16/85	35	130	79	62
Oct. 23/85	40	170	240	97
Oct. 30/85	10	170	49	105
Nov. 13/85	25	170	350	76
Dec. 4/85	70	104	140	76
Sept. 25/85	10	120	-	80
Oct. 2/84	40	126	240	102
Oct. 9/84	30	156	240	88
Oct. 16/84	90	152	350	120
Oct. 17/84	25	120	350	96
Oct. 23/84	15	170	240	104
Oct. 29/84	20	240	180	190
Nov. 5/84	30	180	540	128
Nov. 13/84	25	360	350	190
Dec. 4/84	20	98	170	90
Sept. 24/85	10	75	130	120
Nov. 6/85	40	43	140	37
Nov. 29/85	45	93	140	58
Mean	26.9	144.6	221.6	98.0
Ratio Mean Coliphage to Other means		1:5.4	1:8.2	1:3.6

TABLE 3 Mean microbiological densities and coliphage ratios for various periods of the year, Lemieux Island, Ottawa River, Coliphage-Coliform Study, 1984-1985

Time Period	Coliphage /100 mL	Fecal Coliforms /100 mL MF	Ratio: Coliphage	Fecal Coliforms /100 mL MPN	Ratio: Coliphage	E. coli /100 mL MF	Ratio: Coliphage:	Fecal Streptococci MF /100 mL
Maximum Count Period Sept.-Oct.	25.7	123.4	4.8:1	188.0	7.3:1	93.8	3.6:1	199.7
Minimum Count Period Jan.-May	11.1	53.1	4.8:1	29.5	2.6:1	31.8	2.8:1	33.4
Rest of Samples	18.9	84.8	4.5:1	126.9	6.7:1	52.2	2.7:1	137.4
Samples exceeding 100 fecal coliform/100 mL	26.9	144.6	5.4:1	221.6	8.2:1	98.0	3.6:1	134.3
Duplicate Samples Sept.25-Dec.4/84	33.2	121.1	3.6:1	144.6	4.4:1	72.0	2.2:1	98.3
Sept. 24-Dec. 4/85	26.9	159.5	5.9:1	255.3	9.5:1	105.0	3.9:1	139.3
Survey Mean	18.8	85.0	4.5:1	97.3	5.2:1	57.3	3.0:1	119.6